Amendments to the Specification

Please amend the following paragraphs of the specification by replacement with the following:

The paragraph of page 1, beginning at line 5, ending at the bottom of page 1.

The present invention relates to " DDQ mediated one step dimerisation of dihydro product of toxic β–asarone rich *Acorus calamus* oil towards formation of novel neolignan: 3-ethyl-2-methyl-3-(2′′,4′′,5′′-trimethoxy)phenyl-1-(2′,4′,5′-trimethoxy) phenyl-1-propene" in which 2,4,5-trimethoxyphenylpropane (a dihydro product of asarone obtained via hydrogenation of β–asarone rich *Acorus calamus* oil) of the formula (I), undergoes dimerisation in a single step towards formation of neolignan 3-ethyl-2-methyl-3-(2′′,4′′,5′′-trimethoxy)phenyl-1-(2′,4′,5′-trimethoxy)phenyl-1 propene (named as NEOLASA-I) of the formula II along with biologically active α-asarone and 1-(2,4,5-trimethoxy)phenyl-1-propanone as side products, thereof. Further, neolignan (NEOLASA-I) is hydrogenated to obtain its corresponding dihydro product 3-ethyl-2-methyl-3-(2′′,4′′,5′′-trimethoxy)phenyl-1-(2′,4′,5′-trimethoxy) phenylpropane (named as NEOLASA-II ~~NEONLASA-II~~) so as to confirm the structure as well as to determine the position of double bond existing in the above parent neolignan (NEOLASA-I) which may additionally serve as a simple synthon towards preparation of naturally occurring rare neolignans (such as acoradin or magnosalin or heterotropan and phenyl indane derivative) and their analogues in sufficient quantity to have opportunity for a wide range of biological activities including antifungal, antioxidant, antiinflammatory, neuroleptic, antihepatoxic, anticancer, anti-HIV and anti-PAF activities known for structurally similar neolignan derivatives (such as aurein or hexestrol or nordihydroguaiaretic acid derivatives etc.). In the present invention, the neolignan (NEOLASA-I) formation is the first example of DDQ assisted one step synthesis of neolignan, a dimer of phenylpropanoid, in good yield (32%) from 2,4,5-trimethoxyphenylpropane derivative.

The paragraph beginning at page 2, line 2 and ending at page 5, line 4.

Neolignans and lignans are known for their wide range of biological activities including hapatoprotective, hormone ~~harmone~~ blocking, antibacterial, antifungal, plant growth regulator, anti-HIV, anticancer and antioxidant activities (Macrae, W.D. and Towers, G.H.N., Phytochemistry, 23 (6), 1207-1220 (1984); Ward, R.S., Tetrahedron, 46 (15), 5029-5041 (1990); Charlton, J.L., J. Nat. Prod., 61, 1447-1451 (1998); Alves, C.N.; Barroso, L.P.; Santos, L.S. and

Jardim, I.N, J. Braz. Chem. Soc., 9(6), 577-582 (1998); Juhász, L.; Dinya, Z.; Antus, S. and Gunda, T.E., Tetrahedron Letters, 41, 2491-2494 (2000); Tanaka, T.; Konno, Y.; Kuraishi, Y.; Kimura, I.; Suzuki, T. and Kiniwa, M., Biorg. & Med. Chem. Letts., 12, 623-627 (2002); US patent nos. 6,294,574; 6,201,016; 5.856,323; 5,639,782; 5,530,141; 4,704,462; 4,619,943 and 4,540,709; JP Patent no. 4082837; WO Patent no. 09215294 and EP Patent No. 159565)). Neolignans and lignans are a large group of natural products characterized by the coupling of two C_6-C_3 units which are derived from cinnamic acid derivatives, however, both are present in traces in plants (Rao, K.V. and Rao, N.S.P., J. Nat. Prod., 53(1), 212-215 (1990) and Filler, F.; Bail, J.C.L.; Duroux, J.L.; Simon, A. and Chulia, A.J., Planta Medica, 67, 700-704 (2001)). For nomenclature purposes, the C_6-C_3 unit is treated as propylbenzene and numbered from 1 to 6 in the benzene ring from 7 to 9 (or α to γ) starting from propyl group. With the second C_6-C_3 unit the numbers are primed. When the two C_6-C_3 units are linked by a bond between positions 8 and 8′ (or β and β'), the compound is referred as a lignan. In the absence of the C-8 to C-8′ (or β and β') bond, and where the two C_6-C_3 units are linked by a carbon-carbon bond, compound is referred to as neolignan. Dimers with linkages other than this type are known as cycloneolignan, epoxyneolignan and oxyneolignan etc. Similarly, the presence of a double bond (or triple bond) in the side chain (i.e. C-7 to C-9 or C-7 to C-9) of the lignan, neolignan or epoxyneolignan skeleton is indicated by changing the -ane ending to –ene (or –yne) with a locant to indicate the position of the double bond (Moss, G.P. Pure Appl. Chem., 72 (8), 1493-1523 (2000)). The basic ring system of these neolignans and lignans can be deduced by dimerization of allyl and p-propenylphenols (such as isoeugenol, coniferyl or sinapyl alcohol). Oxidation of phenols often yields phenoxy radicals, which couple with little selectivity. Both C-C and C-O bonds are formed, mainly in ortho- and para- positions to the phenolic hydroxyl. Synthetically useful reactions are obtained only when the reactivity is blocked by substituents in the aforementioned positions. For instance from 2,6- or 2,4-substituted phenols, C-C bonded biphenyls can be obtained in good yields. In other cases coupling can be directed by carrying out the reaction intramolecularly, ring closure being an effective way of inducing regioselectivity (Whitting, D.A. Oxidative Coupling of Phenols and Phenol Ethers. In Comprehensive Organic Synthesis, Trost, B. M.; Fleming, I.; Pattenden, G., Eds.; Pergemon: Oxford, Vol. 3, 659-703 (1991)). Similarly, oxidation of a mixture of two phenols can lead to a mixture of dimers of the individual phenols and cross-coupling products between the different phenols. When one phenol reacts

much faster than the other, for instance if it has a lower oxidation potential, it tends to dimerize without formation of significant amounts of cross-coupling products (Syrjanen, K. and Brunow, G., J. Chem. Soc. Perkin Trans 1, 3425-3429 (1998)). One approach to this problem is to start with the less reactive phenol in large excess, and continuously add the more reactive phenol (and the oxidant) at a rate which is slow enough to keep its concentration too low for significant dimerisation. But this method is cumbersome and leads to a large reaction volumes, and is also difficult to reproduce. A wide range of oxidants such as $K_3Fe(CN)_6$, H_2O_2, $FeCl_3$, VOF_3, thallium (III) tristrifluoacetate, horseradish peroxidase, iodobenzene diacetate (Frank, B. and Schlingloff, G., Liebig. Ann. Chem., 659, 132 (1962); Taylor, W.I. and Battersby, A.R. In "Oxidative Couplings of Phenols", Marcel Dekker, New York (1967); Kametani, T. and Fukumoto, K., Synthesis, 657 (1972); Taylor, E.C.; Andrade, J.G.; Rall, G.J.H. and McKillop, A., J. Am. Chem. Soc., 93, 4841 (1971); Kaisa, S. and Gösta, B., Tetrahedron, 57, 365-370 (2001); Juháasz, L.; Kürti, L. and Antus, S., J. Nat. Prod, 63, 866-870 (2000)) and many others have been used for oxidative coupling but generally these reagents gave poor yield, and often complex mixtures. Indeed, phenoxy radical or phenoxonium ion intermediate is most common for synthesis of lignans and neolignans but there are a few patents and papers where non-phenolic compounds have been used for the synthesis of lignans and neolignans (Kadota, S.; Tsubono, K. and Makino, K., Tetrahedron Letters, 28 (25), 2857-2860 (1987) and Dhal, R.; Landais, Y.; Lebrun, A.; Lenain, V. and Robin, J.P., Tetrahedron, 50 (4), 1153-1164 (1994)). For example, nordihydroguaiaretic acid (one of the most important dimer derived from resinous exudates of many plants), associated with a wide range of pharmacological activities, including the inhibition of the human papillomavirus, herpes simplex, HIV and hyperglycemic activity, has been synthesized by dimerization of non-phenolic compounds such as dimethoxypropiophenone (Perry, C.W. US Patent 3,769,350 (1975)), substituted benzylmagnesium chloride (Akio, M.; Kohei, T.; Keizo, S. and Makoto, K. Tetrahedron Letters, 21, 4017-4020 (1980)) and dimethoxyphenylacetone (Mikail, H.G. and Barbara, N.T. Tetrahedron Letters, 42, 6083-6085 (2001)). However, above methods have a number of disadvantages including special handling of reagents, maintaining temperature below zero degree, expensive reagents and overall low yield, hence, none of the synthetic methods can be scaled up for industrial exploitation. On the contrary, the present invention is free from above drawbacks and discloses one step dimerisation of 2,4,5-trimethoxyphenylpropane (a dihydro product of asarone obtained via hydrogenation of

β–asarone rich *Acorus calamus* oil) of the formula I (Example I) into novel neolignan 3-ethyl-2-methyl-3-(2′′,4′′,5′′-trimethoxy)phenyl-1-(2′,4′,5′-trimethoxy)phenyl-1-propene (named as NEOLASA-I) of the formula II (Example II). Further, neolignan (NEOLASA-I) is hydrogenated to obtain its corresponding dihydro product (3-ethyl-2-methyl-3-(2′′,4′′,5′′-trimethoxy)phenyl-1-(2′,4′,5′-trimethoxy) phenylpropane) (named as NEONLASA-II) (Example III) so as to confirm the structure as well as to determine the position of double bond existing in the above parent neolignan (NEOLASA-I) of the formula (II) which may additionally serve as a simple synthon towards preparation of naturally occurring rare neolignans (such as acoradin or magnosalin or heterotropan and phenyl indane derivative) and their analogues in sufficient quantity to have opportunity for a wide range of biological activities (Wenkert, E.; Gottlieb, H.E.; Gottlieb, O.R.; Pereira, M.O.D.S. and Formiga, M.D., Phytochemistry, 15, 1547-1551 (1976); Kikuchi, T.; Kadota, S.; Yanada, K.; Tanaka, K.; Watanabe, K.; Yoshozaki, M.; Yokoi, T. and Shingu, T., Chem. Pharm. Bull. 31, 1112 (1983); Yamamura, S.; Niwa, M.; Nonoyama, M. and Terada, Y. Tetrahedron Letters, 4891 (1978); Kadota, S.; Tsubono, K.; Makino, K.; Takeshita, M. and Kikuchi, T., Tetrahedron Letters, 28 (25), 2857-2860 (1987); Shimomura, H.; Sashida, Y and Oohara, M., Phytochemistry, 26(5), 1513-1515 (1987); Ahn, B.T.; Lee, S.; Lee, S.B.; Lee, E.S.; Kim, J.G. and Jeong, T.S., J. Nat. Prod., 64, 1562-1564 (2001) and Filleur, F.; Le Bail, J.C.; Duroux, J.L.; Simon, A. and Chulia, A.J., Planta Medica, 67, 700-704 (2001)).

The paragraph beginning at page 12, line 10 and ending at page 13, line 16

β-asarone is experimentally proved to be carcinogenic in animals and has also been found to induce tumors in the duodenal region after oral administration. In addition, β-asarone has also shown chromosome damaging effect on human lymphocytes *in-vitro* after metabolic activation (Taylor, J. M.; Jones, W. I.; Hogan, E. C.; Gross, M. A.; David, D. A. and Cook, E. L., Toxicol. Appl. Pharmacol., 10, 405 (1967); Keller, K.; Odenthal, K. P. and Leng, P. E., Planta Medica 1, 6-9 (1985); Abel, G., Planta Medica, 53(3), 251-253 (1987) and Riaz, M.; Shadab, Q.; Chaudhary, F. M., Hamdard Medicus, 38(2), 50-62 (1995)). As a result, the calamus oil of Asian origin is internationally banned for any kind of use in flavor, perfumery and pharmaceutical industries. To the best of our knowledge, there is no report in which toxic β-asarone of calamus oil is utilized for its value addition except very recently by our group (Sinha, A.K.; Dogra, R. and Joshi, B.P., Ind. J. Chem., 41B, (2002) (in press); Sinha, A.K.; Joshi, B.P. and Dogra, R., Nat.

Prod. Lett., 15(6), 439-444 (2001); Sinha, A.K.; Acharya, R. and Joshi, B.P., J. Nat. Prod. (2002) (in press), Sinha, A.K.; Dogra, R. and Joshi, B.P., Sinha, A.K.; Joshi, B.P., and Dogra, JP Patent Application No. 2001.68716 filed on 12 March (2001); Sinha, A.K.; Joshi, B.P., and Dogra, US Patent Application No. 09-805,832 filed on 14 March (2001) and US Patent Application No. 09-823,123 filed on 31 March (2001)) wherein ammonium formate/palladium-on-charcoal or H_2/palladium-on-charcoal assisted reduction of crude calamus oil containing high percentage of toxic β-asarone provides 2,4,5-trimethoxyphenylpropane (dihydro asarone) of the formula I in 97% purity with yield ranging from 81-87% based on asarones content in calamus oil. Thus, obtained 2,4,5-trimethoxyphenylpropane (or 1-Propyl-2,4,5-trimethoxybenzene) is tested for the first time as five times less toxic than β-asarone and thus, this 2,4,5-trimethoxyphenylpropane enables its application in the products such as mouthwashes, tooth pastes, antiseptic soap products, chewing gum flavors and little in spicy products due to its sweet, ylang, slightly spicy and fruity aroma. In addition, 2,4,5-trimethoxyphenylpropane is also discovered as a simple and an economical starting material for synthesis of a salicylamide based antipsychotic drug (5,6-dimethoxy-N[(1-ethyl-2-pyrrolidinyl)methyl]-3-propylsalicylamide) (Thomas, H.; Stefan, B.; Tomas, D.P.; Lars, J.; Peter, S.; Hakan, H. and Orgen, S. O., J. Med. Chem., 33, 1155-1163 (1990) and Sinha, A.K., US Patent Application No. 09-652376 filed on 31 August (2000)). In the present invention, we have extended the scope of further exploitation of 2,4,5-trimethoxyphenylpropane of the formula I as simple and economical starting material towards the formation of novel neolignan (3-ethyl-2-methyl-3-(2′′,4′′,5′′-trimethoxy)phenyl-1-(2′,4′,5′-trimethoxy)phenyl-1-propene) (named as NEOLASA-I) of the formula II and its dihydro derivative (3-ethyl-2-methyl-3-(2′′,4′′,5′′-trimethoxy)phenyl-1-(2′,4′,5′-trimethoxy)phenylpropane) (named as NEOLASA-II ~~NEONLASA-II~~) of the formula III and side products α-asarone of the formula IIa and 1-2,4,5-trimethoxy)phenyl-1-propanone (isoacoramone) of the formula IIb thereof which are, in fact, biologically active rarer phenylpropanoids.

The paragraph beginning at page 15, line 14, ending at page 16, the fourth line from the bottom.

In order to establish the structure of third crystalline solid having mp 96-97 ^{0}C, a comprehensive investigation of NMR spectral data recorded in two solvents ($CDCl_3$ and DMSO-d_6) for better clarity and separation of each peaks was undertaken. The electrospray (ES)-mass

spectrum of crystalline solid gave molecular ion at m/e 418 (M^+). The ^1H NMR spectra of solid (mp 96-97 ^0C) showed the presence of six methoxyls indicating it to be a possible dimer of asarone like phenylindane (a unsymmetrical dimer reported from *Acorus calamus*) (Saxena, D.B. Phytochemistry 25 (2), 553-555 (1986)) but with change in side chain structure. It is interesting to note from the aromatic region integrated for the four protons indicating that none of aromatic proton participates in dimerisation, however, one of the aromatic proton of phenylindane (2,3-dihydro-4,5,7-trimethoxy-1-ethyl-2-methyl-3-(2,4,5-trimethoxyphenyl)indene) has taken part in dimerisation. The other groups found to be an ethyl group appeared at δ 0.93 (3H, t, H-5), 1.70-1.97 (2H, m, H-4), 3.59 (1H, t, H-3), a tertiary methyl group 1.66 (3H, s, H-6) and a alkene proton on a carbon atom attached to the phenyl ring 6.48 (1H, s, H-1). The above skeleton is further supported by ^{13}C (DEPT-135°) spectra data and mass fragmentation pattern m/z: 418 (M^+) (Example II). On the basis of above spectral data and further, its comparison with some known neolignans such as Magnoshinin, Magnosalin and Heterotropan (Kikuchi, T.; Kadota, S.; Yanada, K.; Tanaka, K.; Watanabe, K.; Yoshozaki, M.; Yokoi, T. and Shingu, T., Chem. Pharm. Bull. 31, 1112 (1983); Yamamura, S.; Niwa, M.; Nonoyama, M. and Terada, Y. Tetrahedron Letters, 4891 (1978) and Kadota, S.; Tsubono, K.; Makino, K.; Takeshita, M. and Kikuchi, T., Tetrahedron Letters, 28 (25), 2857-2860 (1987)) having some degree of similarity in their structure (Wenkert, E.; Gottlieb, H.E.; Gottlieb, O.R.; Pereira, M.O.D.S. and Formiga, M.D., Phytochemistry, 15, 1547-1551 (1976), the crystalline solid is identified as neolignan i.e. 3-ethyl-2-methyl-3-(2′′, 4′′, 5′′- trimethoxy)phenyl-1-(2′,4′,5′-trimethoxy) phenyl-1-propene) (named as NEOLASA-I) (Example II). Further, neolignan (NEOLASA-I) is hydrogenated (Example III) to obtain its corresponding dihydro product i.e. 3-ethyl-2-methyl-3-(2′′,4′′,5′′-trimethoxy)phenyl-1-(2′,4′,5′-trimethoxy)phenylpropane (named as NEOLASA-II ~~NEONLASA-II~~) so as to confirm the structure as well as to determine the position of double bond existing in the above parent neolignan (NEOLASA-I) which may additionally serve as a simple synthon towards preparation of neolignans derivatives in sufficient quantity to have opportunity for a wide range of biological activities including antifungal, antioxidant, anti-inflammatory, neuroleptic, anti-hepatotoxic, anticancer, anti-HIV and anti-PAF activities known for structurally similar neolignan derivatives. Neolignans and lignans comprise a class of natural plant products and they are found in the roots, stems, bark, fruit and seeds of many plant species. More than 200 compounds in this general class have been identified and a great diversity in the

chemical assembly of the two characteristic phenylpropanoid units, as well as degree of oxidation and types of substituents is apparent. In addition, some natural lignans/neolignans are used as starting materials for the semi-synthesis of biological active compounds such as podophyllotoxin, isolated from *Podophyllum* species, is used for the semi-synthesis of the anticancer compounds etoposide and teniposide (Stähelin, H.F. and Wartburg, A.V., Cancer Research, 51, 5-15 (1991)). A number of chemical reviews on natural as well as synthetic neolignans and lignans are available including their biological activities. However, neolignas/lignans are found in traces in plant kingdom and for these reasons, several methods of preparation of neolignas/lignans have been developed by several chemists and some of the reported conventional methods include the following.[[:]]

The paragraph beginning at page 18, line 19 and ending at page 19, line 3.

The β-asarone (6.00 g, 0.029 mol) in 160 ml of ethanol is stirred with 10% palladium on activated charcoal (0.80 g) and ammonium formate (17.00 g, 0.27 mol) at room temperature under nitrogen atmosphere until ~~till~~ the disappearance of starting material. The catalyst was removed by filtration and the solvent was evaporated under reduced pressure. The residue was partitioned between ethyl acetate and water and the ethyl acetate layer washed with water, dried (Na_2SO_4) and filtered. Evaporation of filtrate left a liquid, which was chromatographed, on silica gel using hexane-ethyl acetate mixture with increasing proportion of ethyl acetate up to 10% as the eluent. The eluate was evaporated to give 5.87 g (97%) of a clear sweet and pleasant liquid; R_f 0.69 on silica gel plate (hexane: toluene: ethylacetate = 1:1:0.1) which solidified below 0° C; ^{1}H NMR (DMSO-d6) δ 6.72 (1H, s, H-6), 6.62 (1H, s, H-3), 3.76 to 3.68 (9H, s, 3-OCH_3), 2.5 (2H, t, C-1'), 1.6 (2H, m, C-2') and 0.9 (3H, t, C-3'); ^{13}C NMR ($CDCl_3$) δ 151.4 (C-2), 147.4 (C-4), 142.7 (C-5), 122.7 (C-1), 114.3 (C-6), 98.0 (C-3) and 56.5, 56.2 & 56.0 (3x OCH_3), 31.6 (C-1'), 23.3 (C-2') and 13.79 (C-3'); EIMS m/z 210 (M^+, 39), 181(M^+- C_2H_5, 100), 167 (M^+- C_3H_7, 5), 151 (M^+- OCH_3+ CO, 29), 136 (M^+- C_3H_7 + OCH_3, 10). On the basis of ^{1}H NMR, ^{13}C NMR and Mass spectral data, the above liquid was identified as 2,4,5-trimethoxyphenylpropane in 99 % purity (by GC).

The paragraph at page 19, lines 16-31.

Preparation of 3-ethyl-2-methyl-3-(2′′,4′′,5′′-trimethoxy)phenyl-1-(2′,4′,5′-trimethoxy)phenyl-1-propene: DDQ (6.13-7.97 g) was added over a period of 10-15 min to a ice cold and well stirred solution of 2,4,5-trimethoxyphenylpropane (5.67g, 0.027 mol) in acetic acid (55 mL) and stirring was continued at room temperature for over night The precipitated solid of $DDQH_2$ was filtered and the filter cake washed twice with acetic acid. In addition to acetic acid, other organic acids, such as propionic acid, may be used. The combined acetic acid layer was evaporated and mixture was poured into water and extracted with dichloromethane (3 x 70 mL). In addition to dichloromethane, other aliphatic halogenated hydrocarbons, such as carbontetracholride or chloroform, may be used. The combined organic layer were washed with brine (3 x 15 mL), 10% sodium bicarbonate (2 x 10 mL), brine (3 x 15 mL) and dried over sodium sulphate. The residue obtained on evaporation of the solvents was chromatographed on silica gel using hexane-ethyl acetate mixture with increasing proportion of ethyl acetate upto 40% and the fractions having similar R_f were mixed which after evaporation of solvents provided three viscous liquids which were further crystallized from mixture of hexane and methanol to afford three white solids having mp 44-45^{0}C, 109-110^{0}C and 96-97^{0}C with 9%, 22% and 32% yield respectively.